

Heat Resistance of Salmonellae in Concentrated Milk

C. A. DEGA, J. M. GOEPFERT, AND C. H. AMUNDSON

Food Research Institute, Department of Bacteriology, and Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706

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The heat resistance of *Escherichia coli*, *Salmonella typhimurium*, and *Salmonella alachua* in milk solutions containing 10, 30, 42, and 51% (w/w) skim milk for total solids was determined. Increased milk-solids level effected a significant increase in the heat resistance of each organism. Although *E. coli* was more heat-resistant than both strains of *Salmonella* in 10% milk, the situation was reversed in 42 and 51% milk. Prior growth temperature was found to exert a profound effect on the heat resistance of *S. typhimurium*. Growth of *S. typhimurium* in 42% milk solids for 24 hr did not greatly enhance the thermal resistance of the organism when heated in a fresh 42% solids concentrate. Application of a partial vacuum during heating greatly diminished the decimal reduction times of *S. typhimurium* and *E. coli* and, in addition, virtually eliminated the protective effect of increased solids level.

It is well known that bacterial cells are more resistant to dry heat than moist heat. There are several papers that amply demonstrate an increase in bacterial heat resistance as the solute concentration of the heating menstruum increases (1, 2, 11, 14, 15). This increase in resistance has been suggested to be a consequence of reduced water activity, and, undoubtedly, this is an important factor. However, Goepfert et al. (11) reported that the chemical nature of the solute controlling the water activity was more influential on the heat resistance of salmonellae at water activity levels above 0.75. This was later confirmed by Baird-Parker et al. (1) and Moats et al. (14). Because of this, it becomes necessary to experimentally determine the heat resistance of salmonellae in each individual test material rather than extrapolating data derived from experiments on similar but nonidentical products.

In 1968, McDonough and Hargrove (13) reported that survival of two species of *Salmonella* was greater in concentrated milk (50% solids) than in skim milk heated at the same temperature. This study was undertaken to extend the observations of McDonough and Hargrove and to investigate the factors (growth temperature, growth medium, reduced pressure) that influence the heat resistance of salmonellae in a single food material, i.e., concentrated skim milk. It was hoped that the

data generated by this study would enable the dry milk industry to assess their current practices with regard to efficiency of the process in destroying salmonellae and to add to present knowledge about the heat resistance of salmonellae in dry and semidry environments.

MATERIALS AND METHODS

Bacterial cultures. All cultures in this investigation, i.e., *Salmonella typhimurium*, *Salmonella alachua*, and *Escherichia coli* (0104:H7), were obtained from the culture collection of the Food Research Institute, University of Wisconsin. The strain of *S. alachua* had been originally isolated from nonfat dry milk. Stock cultures were stored at room temperature on nutrient agar slants (NA) in screw-cap tubes. Working cultures were transferred every 24 hr in Trypticase soy broth (TSB) and incubated without agitation at 35 to 37 C, except as noted below in the study of the effect of growth medium and temperature on the heat resistance of cells.

Milk solutions. Skim and concentrated milk solutions were prepared from nonfat dry milk powder as described previously (6).

Heating of microorganisms. (i) When heated at atmospheric pressure, milk solutions at solids levels of 10, 30, 42, and 51% (w/w) were placed in stainless-steel mixing cups. The cups were placed in a water bath at test temperature in such a manner that the level of the bath was 2 to 3 inches (ca. 5.1 to 7.6 cm) above the level of the milk in the cup. After equilibration of the milk solution to the test temperature, the inoculum (24-hr-old TSB-grown cells) was added. After inoculation and throughout the trial,

the heating menstruum was agitated by a mechanical stirrer. Test solution and water bath temperature were also monitored throughout the trial.

(ii) When heated under reduced pressure, milk solutions were placed in the laboratory scale vacuum pan described previously (7). After equilibrating to the test temperature and pressure, the inoculum (1 to 2%, v/v) was introduced through the port designed for this purpose. Throughout the trial, the inflow of pretempered, sterile, distilled water was matched to the outflow of condensate to maintain a constant solids level.

Enumeration of microorganisms. In most trials, survivors were enumerated by surface-plating procedures. At appropriate intervals, 1-ml samples were taken from the heating menstruum and added to 9 ml of 0.1% peptone-water. One-tenth milliliter quantities of the appropriate peptone-water dilutions were surface plated on Trypticase soy agar supplemented with 0.2% yeast extract. Plates were examined after incubation at 35 to 37 C for 48 hr.

When the inoculum was grown in concentrated milk prior to heating in a fresh concentrate, the three-tube most-probable-number (MPN) procedure was employed to enumerate survivors. This entailed pre-enriching 1-ml samples of the appropriate dilutions in nutrient broth for 6 hr at 35 to 37 C prior to transferring 1-ml portions to tubes containing 9 ml of tetrathionate broth and selenite-cystine broth. The enrichment media were incubated at 35 to 37 C for 24 hr. One loopful of each broth was streaked onto *Salmonella-Shigella* agar plates which were then incubated at 35 to 37 C for 48 hr. Typical colonies were confirmed as salmonellae by appropriate serological tests. MPN values were calculated on the basis of the pattern of positive dilutions in the series.

Calculation of D values. The number of survivors were plotted (ordinate) against time (abscissa) on semi-logarithmic graph paper. All trials were conducted for a period of time sufficient to result in a 5-log cycle drop in viable cells. In some instances, the plots were diphasic, i.e., there was an initial phase of rapid death followed by a phase in which death proceeded at a slower rate. In such cases, the decimal reduction time (D) value was obtained from the portion of the plot describing the slower rate. Instances in which diphasic curves were obtained are so indicated in Table 1.

RESULTS AND DISCUSSION

Previously, McDonough and Hargrove (13) showed that growth of salmonellae occurred in milk concentrates containing 60% solids. We confirmed these observations and delineated the temperature limits within which salmonellae would grow in milk concentrates (6). Thus, it was decided to investigate the processing parameters that would suffice to ensure the destruction of salmonellae in concentrated milk.

The results of the trials in which *S. typhi-*

murium, *S. alachua*, and *E. coli* (all grown at 35 to 37 C) were heated in milk solutions are shown in Table 1. It is clearly evident that increased solid levels result in an increased resistance to heat destruction by all of the organisms tested. This was not unexpected and confirms the earlier observations of McDonough and Hargrove (13). The basis for the increased resistance to heat is not known. It is unlikely that lactose is affording protection since Fay (9) reported no increase in heat resistance when cells were heated in concentrated-lactose solutions. We have confirmed (*unpublished data*) this observation. Moats et al. (14) reported that casein added to phosphate buffer did not significantly protect *S. anatum* from heat destruction, but the concentration of casein was low (i.e., 1%); these data tell us little regarding more concentrated casein solutions. Kadan et al. (12) reported that addition of fat (up to 14%) did not influence the heat resistance of *Staphylococcus aureus* in skim milk. However, the addition of 30% serum solids did effect approximately a 45% increase in the D_{60C} value for staphylococci in skim milk. Further investigation is needed to determine the nature of the substance(s) in skim milk that is responsible for the increased heat resistance of salmonellae and escherichiae in concentrated milk.

The relative heat resistance of the test organisms was found to vary with the solids level of the heating menstruum. Thus, although the strain of *E. coli* was more resistant than both strains of salmonellae in 10% milk, the situation was reversed in the 42 and 51% solids milk. The consequence of the relative heat resistance values in concentrated milks is that a heat treatment given these products might render the product coliform-free but leave it contaminated with *Salmonella*. Therefore, the value of a coliform test as an indicator of enteric contamination in concentrated milk is rather minimal. The relative susceptibility of *E. coli* and salmonellae to spray drying must be determined before the value of the indicator organism analyses can be accurately assessed. Such an investigation is currently in progress.

It was interesting to note that *S. alachua* was more heat-resistant in concentrated milk than *S. typhimurium*. It would be easy to speculate that perhaps this resistance enabled the *S. alachua* strain to survive the processing treatment inherent in the manufacture of the dry milk product from which it was isolated. Without additional information, this would only be speculation. However, a correlation between resistance to environmental condi-

TABLE 1. Influence of milk solids concentration on the thermal resistance of *Escherichia coli*, *Salmonella typhimurium*, and *S. alachua* grown in Trypticase soy broth at 37 C

| Organism | 10% Solids | | 30% Solids | | 42% Solids | | 51% Solids | |
|-----------------------|-----------------------|---------------------------------|-----------------------|--------------------|-----------------------|--------------------|-----------------------|--------------------|
| | Temp (C) ^a | Mean D ^b value (min) | Temp (C) ^a | Mean D value (min) | Temp (C) ^a | Mean D value (min) | Temp (C) ^a | Mean D value (min) |
| <i>S. typhimurium</i> | 57.1 | 1.4 | 58.0 | 2.5 | 60.8 | 2.9 | 65.0 | 1.7 |
| | 56.8 | 2.0 | 55.0 | 11.0 ^c | 59.6 | 4.1 | 62.8 | 3.8 |
| | 55.7 | 3.2 | 51.7 | 59.8 ^c | 58.8 | 5.4 | 62.3 | 4.5 |
| | 55.2 | 4.7 | | | 58.5 | 5.9 | 61.0 | 6.7 |
| | 54.7 | 7.5 | | | 57.0 | 9.9 | 59.2 | 10.4 |
| | 53.9 | 10.8 | | | 55.1 | 18.3 ^c | 57.0 | 26.6 |
| | 52.9 | 20.0 | | | | | | |
| | 52.5 | 22.5 | | | 53.8 | 40.0 ^c | | |
| | 51.4 | 49.0 | | | 52.8 | 48.5 ^c | | |
| | <i>E. coli</i> | 58.3 | 1.8 | 58.0 | 2.4 | 59.6 | 2.3 | 62.8 |
| 57.0 | | 2.4 | 55.0 | 11.0 ^c | 59.3 | 3.0 | 60.7 | 4.2 |
| 56.8 | | 2.9 | 51.7 | 49.3 ^c | 58.8 | 4.3 | 59.2 | 7.5 |
| 56.6 | | 3.3 | | | 58.6 | 5.0 | 59.0 | 7.9 |
| 55.2 | | 7.3 | | | 57.1 | 7.7 | 57.0 | 13.5 |
| 54.8 | | 9.6 | | | 55.1 | 13.5 ^c | 55.3 | 18.3 ^c |
| 54.3 | | 13.9 | | | 54.8 | 17.5 ^c | 55.0 | 23.5 ^c |
| 53.7 | | 16.8 | | | 53.4 | 25.0 ^c | | |
| 53.5 | | 18.7 | | | 53.0 | 29.2 ^c | | |
| 52.4 | | 33.3 | | | | | | |
| <i>S. alachua</i> | 59.2 | 0.5 | | | 61.1 | 3.0 | 64.0 | 2.8 |
| | 57.8 | 1.1 | | | 59.7 | 4.3 | 63.0 | 4.8 |
| | 57.0 | 1.6 | | | 58.7 | 5.9 | 60.0 | 13.5 |
| | 56.7 | 2.5 | | | 56.9 | 12.5 | 59.0 | 18.0 |
| | 55.3 | 5.3 | | | 55.0 | 21.6 ^c | 58.5 | 18.0 |
| | 55.0 | 6.2 | | | 53.3 | 41.7 ^c | 58.0 | 21.0 |
| | 54.6 | 6.5 | | | | | 57.2 | 33.0 |
| | 54.5 | 7.7 | | | | | 56.7 | 38.0 |
| | 54.1 | 9.5 | | | | | | |
| | 54.0 | 10.5 | | | | | | |
| | 53.1 | 16.3 | | | | | | |
| | 53.0 | 20.4 | | | | | | |

^a Values, \pm 0.2 C.

^b Decimal reduction time; represents an average of 2 to 5 trials at each temperature.

^c Diphasic curves.

tions and frequency and source of isolation has been suggested (8) and may in fact warrant further attention.

Figure 1 shows the thermal destruction curves for *S. typhimurium* (grown at 35 to 37 C) heated in the various milk solutions. It can be seen that the Z value increases as the solids level in the milk is increased. The same behavior was noted for *E. coli* (Z = 4.6, 4.9, 6.3, and 7.9 C at 10, 30, 42, and 51% solids, respectively) and *S. alachua* (Z = 4.1, 6.2, and 6.9 C at 10, 42, and 51% solids, respectively). An increase in Z value was previously reported by Goepfert and Biggie (10) in their investigation on the heat resistance of salmonellae in chocolate. Similarly, Cotterill and Glauert (3) noted

a Z value of 9.1 C for *S. oranienburg* heated in egg yolk containing 10% NaCl. This behavior underscores the necessity for determining the heat resistance of an organism in situ rather than extrapolating from data derived in experiments conducted using laboratory media or dilute-food suspensions.

In a study of the heat resistance of salmonellae in laboratory media, Ng et al. (16) reported a profound influence of prior (to heating) growth temperature on the heat resistance of two strains of salmonellae. The influence of growth temperature on the heat resistance of *S. typhimurium* in 10 and 42% solids-containing milk solutions was investigated. Figure 2 shows the thermal destruction curves

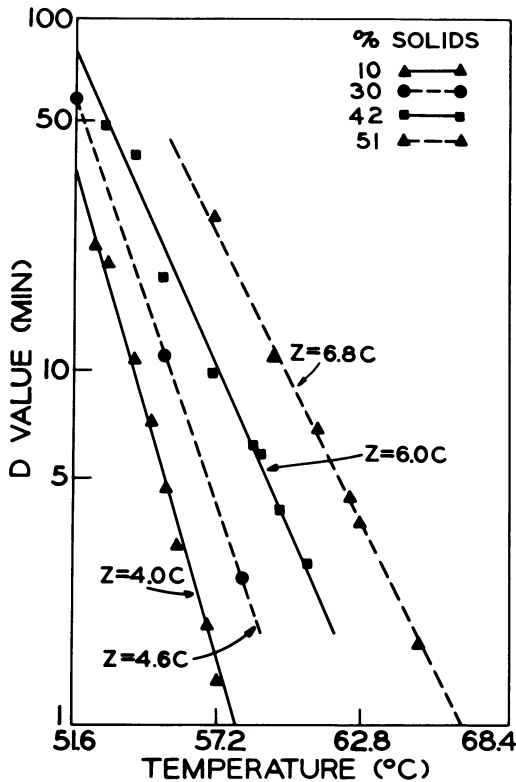


FIG. 1. Influence of milk solids levels on the thermal destruction curves of *S. typhimurium* grown at 37 C.

for *S. typhimurium* grown in TSB at 22, 37, and 43 C prior to heating in the milk solutions. In all cases, the salmonellae grown at higher temperatures were more resistant than those grown at low temperature. The effect of prior growth temperature was greater on cells heated in 10% milk than in 42% milk. These data and those of Ng et al. (16) would indicate that, to ensure a safety factor in determining processing parameters designed to destroy salmonellae, stationary-phase cells grown at or near the maximum growth temperature for the organism should be employed as the test inoculum. This is particularly important for those industries in which salmonellae might have the opportunity for growth at elevated temperatures either in in-line product or the environment.

Several investigators have reported that prior growth of microorganisms in or exposure to certain concentrated (or reduced water activity) solutions has resulted in increased resistance when exposed to heat in a concentrated solution. For example, Fay (9) noticed that the resistance of *E. coli* to heat destruc-

tion increased when cells were incubated for various periods in 50% sucrose prior to heating. Similarly, Calhoun and Frazier (2) reported that *Pseudomonas fluorescens* grown in broth containing glucose ($a_w = 0.97$) was more resistant to heating in reduced (by glucose) water activity solutions. Goepfert et al. (11) found that prior growth of *S. montevideo* in 10% glycerol broth increased the heat resistance of cells in concentrated glycerol solution. More recently, Cotterill and Glauert reported similar behavior for *S. oranienburg* held in either TSB or egg yolk (4) containing NaCl prior to heating. To evaluate the effect of prior growth of salmonellae in concentrated milk on their survival when heated in a menstruum of identical solids concentration, the following experiment was performed. *S. typhimurium* was inoculated into 42% milk and incubated at 35 to 37 C for 24 hr. At that time, a sample of this medium was used to inoculate (1 to 2%, v/v) a fresh (pretempered to 55.1 C) 42% solids solution. Samples were taken periodically, and

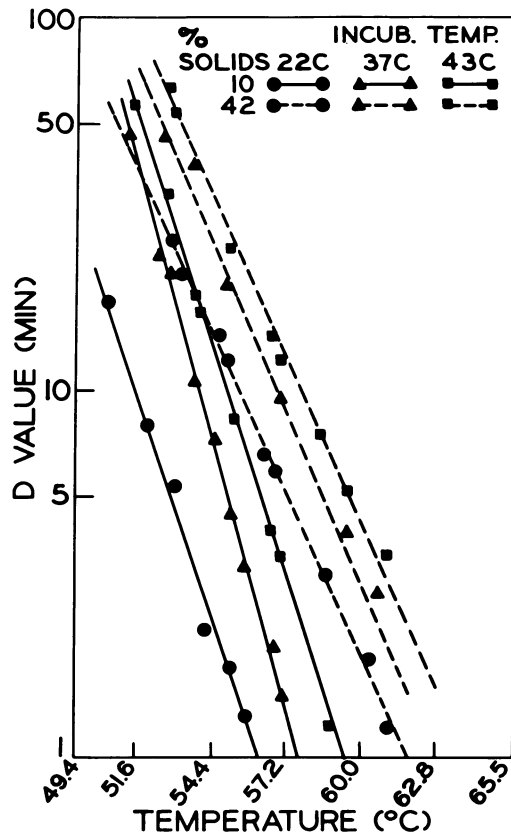


FIG. 2. Influence of growth temperature on the thermal resistance of *S. typhimurium* in 10 and 42% milk solids.

surviving salmonellae were enumerated by the MPN procedure. A mean $D_{55.1\text{ C}}$ value of 20.0 min for cells so treated was obtained. Comparing this with a $D_{55.1\text{ C}}$ value of 18.3 min for cells grown in TSB at 35 to 37 C would indicate that the heat resistance of this one strain was not significantly enhanced by growth in 42% solids-containing milk solution. However, Fay (9) reported that only brief exposure to 50% sucrose was necessary to enhance the heat resistance of *E. coli*, and his data indicate that protracted exposure (i.e., >7 hr) resulted in a return to normal resistance. Cotterill and Glauert (4) reported that increase in thermal resistance of *S. oranienburg* which occurred during storage in egg yolk containing 10% NaCl was temperature- and time-dependent. Their data show that maximum thermal resistance was attained after 12 to 24 hr at 32 C and at 12 hr at 40 C. At 40 C, the thermal resistance after 24 hr of storage was nearly equivalent to that possessed by salmonellae that were not exposed to the salt-containing yolk prior to heating. Interestingly, prior exposure to yolk containing 10% sugar did not enhance the thermal resistance of the salmonellae that were heated in this product. It is therefore possible that the very similar thermal resistance of TSB-grown and 42% milk-grown *S. typhimurium* is due to (i) the absence of any effect due to prior exposure or (ii) too long an exposure to the concentrated milk prior to heating. Experimentation employing cells that were exposed to the concentrate for shorter periods of time prior to heating would demonstrate whether an enhancement of heat resistance followed by a return to normal resistance was actually occurring. It is also possible that concomitant growth by the microflora of the milk influenced the heat resistance of the salmonellae.

This could only be negated by employing a sterile product in the experiment.

In a previous paper (6), we reported that vacuum concentration of skim milk was lethal to salmonellae and *E. coli* only when the vapor temperature exceeded the maximum growth temperature of the organisms. These observations were extended to compare the heat resistance of *S. typhimurium* and *E. coli* in 10 and 30% milk solids heated at atmospheric pressure and under reduced pressure. The data are shown in Table 2. It is quite evident that reducing the pressure is an effective means of reducing the heat treatment necessary to destroy salmonellae in concentrated milks. Apparently, not only was the D value of each organism reduced, but the protective effect of higher solids concentration was also minimized.

Similar effects of reduced pressure on the heat resistance of salmonellae were noted by Ballas (cited in reference 5). His research resulted in USDA acceptance of an alternate method of pasteurizing egg whites. In this process, 17 to 20 inches of vacuum are applied to the liquid whites prior to heating to 56.7 C for 3.5 min. The explanation for this reduced heat resistance under partial vacuum is not known but is of significant importance to merit further examination.

It is apparent that there are a number of factors that affect the heat resistance of enteric bacteria in food products. It is also clear that these factors cause significant differences in the behavior of salmonellae in broth and food menstrua. Experience has taught us the futility of attempting to predict behavior of salmonellae in food products based on data derived from experiments performed in laboratory media and buffers. It is hoped that more

TABLE 2. Comparison of the thermal resistance of *Escherichia coli* and *Salmonella typhimurium* in milk solutions heated at atmospheric pressure and under reduced pressure

| Organism | Temp C ^a | D value ^b (min) in 10% milk solids heated at | | D value (min) in 30% milk solids heated at | |
|-----------------------|---------------------|---|------------------|--|------------------|
| | | Atmospheric pressure | Reduced pressure | Atmospheric pressure | Reduced pressure |
| <i>S. typhimurium</i> | 54.4 | 7.9 | 2.8 | 14.8 ^c | |
| | 53.5 | 13.7 ^c | | 24.3 ^c | 4.4 |
| | 51.7 | 43.0 | 9.1 | 59.8 | 9.9 |
| <i>E. coli</i> | 53.5 | 18.7 | 7.1 | 21.1 ^c | 6.6 |
| | 51.7 | 49.2 ^b | 11.8 | 49.3 | 11.6 |

^a Values, ± 0.2 C.

^b Decimal reduction time.

^c Data obtained by extrapolating from thermal destruction curves, Fig. 2.

studies of salmonella behavior in situ will be performed so that data are available to enable food processors to design adequate processing schedules to destroy salmonellae.

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